ALLIA.143A PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	H. Zaghouani	Group Art Unit 1644
Appl. No.	:	08/779,767	
Filed	:	January 7, 1997	
For	:	COMPOUNDS, COMPOSITIONS AND METHODS FOR THE ENDOCYTIC PRESENTATION OF INNUMOSUPPRESSIVE FACTOR	
Examiner	:	P. Nolan	

I hereby certify that this correspondence and all marked attachments are being deposited with the United States Postal Service as first-class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on

October 19, 2000
(Date)

Daniel Hart

Daniel Hart, Reg. No. 40,637

DECLARATION UNDER 37 C.F.R §1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

1. This Declaration is being submitted to demonstrate that the elimination of the symptoms of autoimmune disease upon treatment with the compositions claimed in the above-identified application is not a consequence of increased half-life of the claimed compositions, that the compositions claimed in the above-identified patent application permanently eliminate the symptoms of autoimmune disease, that neither the compositions disclosed in Bona nor the compositions disclosed in Kuchroo are capable of inactivating T cells as do the compositions claimed in the above-identified patent application, and that the immunoglobulins comprising a T cell receptor antagonist are generally effective in treating autoimmune diseases.

2. I am an inventor on the above-identified patent application and am familiar with the specification and prosecution history.

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3. I am skilled in the fields of immunology and molecular biology as evidenced by the curriculum vitae which accompanied the Declaration submitted on March 29, 2000 in the above-identified application.

4. The elimination of the symptoms of autoimmune disease upon treatment with the compositions claimed in the above-identified application is not a consequence of increased half-life of the claimed compositions. As indicated in Exhibit B of the Declaration submitted March 29, 2000, mice suffering from EAE fully recovered from this autoimmune disease within 50 days of treatment with the claimed compositions and did not relapse for the duration of the experiment (120 days from the administration of the claimed compositions). The experiment of Exhibit B of the Declaration submitted March 29, 2000 demonstrate that although a period of 15 weeks elapsed between the last administration of the compositions claimed in the above-identified application and the termination of the experiment, the mice did not develop disease symptoms.

5. The effectiveness of treatment with the compositions claimed in the above-identified application is unlikely to be a result of increased half life resulting from inseration of the T-cell antagonist into an immunoglobulin backbone. The half life of immunoglobulins in 6-8 week old mice such as those used in the experiment of Exhibit B of the Declaration submitted March 29, 2000 is on the order of 4.5 days. (See Takemori et al. Immunological Review 79: 103-117 (1984), provided herewith as Exhibit A). Thus, since the amount of immunoglobulin present in the subjects after 15 weeks is negligible (approximately 2^{25} times lower than the originally administered amount), it is unlikely that a sufficient amount of the composition remains to provide direct protection. Rather, as discussed in more detail below, the effectiveness of the claimed compositions is most likely due to a permanent inactivation of the T cells directed against the antigen responsible for the autoimmune disease.

6. The compositions claimed in the above-identified patent application permanently elimated the symptoms of autoimmune disease in subjects which were suffering from autoimmune disease prior to the administration of the claimed compositions.

The mice treated with the claimed composition in Exhibit B of the Declaration submitted March 29, 2000 were approximately 2 months old at the time the claimed compositions were administered. Thus, at the conclusion of the experiment (120 days from the administration of the claimed compositions) the mice were approximately six months old. As indicated in Endoh, M. et al., Journal of Neuroimmunology 29: 21-27, which is provided herewith as Exhibit B, mice

have poor susceptibility to EAE once they reach the age of 24-27 weeks (6 months). Accordingly, the fact that the mice treated with the claimed compositions did not exhibit disease symptoms at 6 months of age indicates that they were permanently cured of the disease, since at that age they are no longer susceptible to the disease.

7. Neither the compositions disclosed in Bona nor the compositions disclosed in Kuchroo are capable of inactivating T cells as do the compositions claimed in the above identified patent application. The compositions of the present invention inactivate T cells directed against proteolipid protein as follows. Immnunoglobulins containing a T cell receptor antagonist derived from the proteolipid protein are internalized into antigen presenting cells via their interaction with the Fc receptor. Inside the cell, the immunoglobulins move to the endosomes where antagonist peptides are cleaved from the immunoglobulin and bind to newly synthesized MHC molecules. The complexes between the antagonist peptides and the MHC molecules move to the cell surface where they engage autoreactive T cells. The interaction between the antagonist/MHC complexes and the autoreactive T cells reduces cytokine production, thereby inactivating autoreactive T cells.

This mechanism of action is documented in the accompanying article by Legge et al., J. Exp. Med. 185: 1043-1053 (1997) provided herewith as Exhibit C. Experiments using immunoglobulins containing the T cell receptor antagonist PLP-LR demonstrated that these compositions reduced proliferation of T cells *in vitro* (See pages 1047-1048 and Figure 5 of Exhibit C) and *in vivo* (See pages 1048 and Figure 8 of Exhibit C).

Furthermore, the inactivation of T cells does not occur by a competitive mechanism in which the T-cell antagonist occupies MHC Class II molecules and prevents the antigenic peptide from binding thereto. This is evidenced by the fact that immunoglobulins containing the PLP-2 peptide, which is not a T cell receptor antagonist, inserted therein did not inactivate T cells, while immunoglobulins containing the T cell receptor antagonist PLP-LR did inactivate T cells. (See page 1048 and Figure 6 of Exhibit C).

The compositions which had been actually prepared and which were discussed in Bona were compositions in which an immunogenic peptide was inserted into an immunoglobulin backbone. Thus, the goal of these compositions was to stimulate an immune response (i.e. activate T cells) rather than to inhibit an immune response (i.e. inactivate T cells) as do the compositions of the present invention.

While Bona speculates that self antigens could be introduced into immunoglobulin backbones, there is no mention of inserting T cell antagonists into the immunoglobulin backbone. Furthermore, in Bona's discussion of self antigens he states "In the later case cases, it is possible that the Ig bearing epitopes of self antigen will be more efficient for peptide competition therapy envisioned as a novel therapeutic approach of autoimmune disease." This statement indicates that Bona hypothesized that the speculated immunoglobulins containing self antigens would be internalized into the cells, bind MHC proteins inside the cells, and be transported to the surface of the cells such that all MHC proteins on the surface of the cells would be occupied by the self antigen and would be unavailable for binding the pathogenic peptide. This mechanism is unlikely to work because MHC molecules and pathogenic peptides are synthesized continuously in unlimited amounts. Accordingly, in contrast to Bona's hypothesis, complexes between the MHC molecules and the pathogenic peptides would in fact be formed and translocate to the surfaces of the antigen presenting cells. Bona's hypothesis was ruled out in Figure 6 of Exhibit C. Accordingly, Bona did not conceive of the above mechanism whereby immunoglobulins containing T cell antagonists (note that Bona mentions immunoglobulins containing antigens or self antigens but makes no mention of immunoglobulins containing T cell antagonists) inactivate T cells.

The compositions disclosed in Kuchroo are incapable of inactivating T cells. Kuchroo discloses peptides which function as T cell receptor antagonists. Since the peptides of Kuchroo are not embedded in an immunoglobulin backbone, they are not internalized via the mechanism described above with respect to the compositions of the present invention. Accordingly, the peptides of Kuchroo cannot bind to newly synthesized MHC molecules to inactivate T cells via the mechanism utilized by the compositions of the present invention.

8. Immunoglobulins containing T cell receptor antagonists derived from proteins other than proteolipid protein were also effective in treating autoimmune disease. In particular, the following experiment demonstrates that immunoglobulins containing a T cell receptor antagonist derived from myelin basic protein (MBP) were effective in suppressing experimental allergic encephalomyelitis (EAE) induced by MBP87-99 peptide.

The amino acid sequence 87-99 (VHFFKNIVTPRTP) of myelin basic protein (MBP87-99) is encephalitogenic and induces experimental allergic encephalomyelitis when injected into SJL/J mice emulsified in complete Freund's adjuvant (CFA). The EAE induced by MBP87-99

is less severe than that induced by proteolipid protein. EAE induced by MBP87-99 is characterized by a single episode of disease followed by complete recover as opposed to the relapse and remitting EAE induced by proteolipid protein.

For simplicity purposes MBP87-99 peptide is referred to herein as MBP3 peptide. An altered peptide was generated from MBP3 by substituting the proline in position 96 with alanine. This altered peptide, designated MBP3A (VHFFKNIVTARTP), functions as a T cell antagonist and suppresses passive disease transferred into mice by a pathogenic T cell clone specific for MBP3 (Brock et al., Nature 379: 343-346 (1996), provided herewith as Exhibit D).

A nucleotide sequence encoding MBP3A was inserted into the variable region of the 91A3 immunoglobulin heavy chain and this chimeric heavy chain was transfected into SP2/0 B cell line along with a nucleotide sequence encoding the parental 91A3 immunoglobulin light chain. The resulting immunoglobulin containing the MBP3A peptide therein, designated Ig-MBP3A, was then purified and tested for suppression of EAE induced by MBP3 peptide as follows.

Seven week old SJL/J mice were induced for experimental allergic encephalomyelitis (EAE) with MBP3. Induction of EAE was carried out by subcutaneous injection in the footpads and at the baso of the limbs and tail with a 200 µl incomplete Freund adjuvant (IFA)/PBS (v/v) solution containing 200 µg *Mycobacterium tuberculosis* H37Ra and 100 µg of free MBP3 peptide. Six hours later the mice were given intravenously 5×10^9 inactivated *Bordetella pertussis* (Bioport, Lansing, MI). A second injection of *B. pertussis* was given after 48 hours. Subsequently, the mice were scored daily for clinical signs of EAE as follows: 0, no clinical score; 1, loss of tail tone; 2, hindlimb weakness; 3, hindlimb paralysis; 4, forelimb paralysis; and 5, moribund or death.

When sign of paralysis became apparent one group was treated with Ig-MBP3A (indicated by circles in Exhibit E provided herewith), another group with the control Ig-W not including any MBP peptide (indicated by squares in Exhibit E), and a third group was left untreated (Nil, indicated by triangles in Exhibit E). The mice were then scored daily for clinical signs of EAE. Each point represents the mean clinical score of 7 mice. As indicated in Exhibit A, Ig-MBP3A is the Ig chimera carrying the partial antagonist peptide, MBP3A, and Ig-W is the parental Ig not carrying any PLP or other peptide which was used as a control. The treatment

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consists of three intraperitoneal injections of 200 μg of Ig-MBP3A or Ig-W on days 11, 16, and 21 post disease induction totaling 600 μg for all injections.

As indicated in Exhibit E, the untreated mice developed a mild monophasic EAE characteristic of disease induced with MBP3 peptide. The mice treated with the control Ig-W developed clinical paralysis similar to the untreated mice. However, treatment with Ig-MBP3A prevented the disease from taking a normal course and the mice did not suffer symptoms more severe than exceed a mild loss of tail tone for the entire 50 day period of clinical assessment. These results indicate that delivery of immunoglobulins containing T cell receptor antagonists therein down-regulated pathogenic T cells and that such effectiveness is not unique to immunoglobulins containing a Tcell receptor antagonist derived from proteolipid protein but is a general property of immunoglobulins containing T cell receptor antagonists.

9. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or patent issuing therefrom.

Dated: October 19, 2000

By: _____
Habib Zaghouani

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